

Applicant : Melvin et al.
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Attorney's Docket No.: 12489-003002 / UMMC Ref.:
UMMC 96-01

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 12489-003002.

Respectfully submitted,

Date: March 15, 2002

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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 17, line 3, has been amended as follows:

Reverse transcription polymerase chain reaction (RT-PCR) experiments to detect CYP1B1 mRNA were carried out as described in McKay et al (23). RNA was extracted from tissue samples and cDNA was synthesised from the isolated RNA using oligo (dT). The CYP1B1 primers had the following sequences: Forward 5'-AAC TCT CCA TCA GGT GAG GT-3' (SEQ ID NO:3) (nt 2104-2123); Reverse 5'-TAA GGA AGT ATA CCA GAA GGC-3' (SEQ ID NO:4) (nt 2573-3593) giving a PCR product of 489bp. β -actin was used as a positive control to confirm the presence and integrity of mRNA in each sample and the β -actin primers which were bought from Stratagene (Cambridge, UK) had the following sequences: Forward 5'-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3' (SEQ ID NO:5) (nt 1067-1105); Reverse 5'-CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG-3' (SEQ ID NO:6) (nt 1876-1905). PCR with 35 cycles of amplification for both CYP1B1 and β -actin was performed as described (23). The positive control for CYP1B1 was a 2.78 kb CYP1B1 cDNA and the negative control was sterile water in place of cDNA. After PCR 10 μ l of the PCR product was electrophoresed on a 1.5% agarose gel which incorporated 0.007% w/v ethidium bromide and visualised by UV illumination. The CYP1B1 PCR product was sequenced, after purification, by the direct dideoxy sequencing technique with a T7 sequencing kit (Pharmacia, Milton Keynes, UK) used according to the manufacturer's protocol. To further investigate the relative amount of CYP1B1 mRNA in normal and tumour tissues, semi-quantitative RT-PCR of normal and tumour kidney samples was performed using serial dilution of cDNA (24). β -actin mRNA was used as an internal control (29).

Replace the paragraph beginning at page 17, line 3, with the following rewritten paragraph:

--Reverse transcription polymerase chain reaction (RT-PCR) experiments to detect CYP1B1 mRNA were carried out as described in McKay et al (23). RNA was extracted from tissue samples and cDNA was synthesised from the isolated RNA using oligo (dT). The CYP1B1 primers had the following sequences: Forward 5'-AAC TCT CCA TCA GGT GAG GT-3' (SEQ ID NO:3) (nt 2104-2123); Reverse 5'-TAA GGA AGT ATA CCA GAA GGC-3' (SEQ ID NO:4) (nt 2573-3593) giving a PCR product of 489bp. β -actin was used as a positive control to confirm the presence and integrity of mRNA in each sample and the β -actin primers which were bought from Stratagene (Cambridge, UK) had the following sequences: Forward 5'-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3' (SEQ ID NO:5) (nt 1067-1105); Reverse 5'-CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG-3' (SEQ ID NO:6) (nt 1876-1905). PCR with 35 cycles of amplification for both CYP1B1 and β -actin was performed as described (23). The positive control for CYP1B1 was a 2.78 kb CYP1B1 cDNA and the negative control was sterile water in place of cDNA. After PCR 10 μ l of the PCR product was electrophoresed on a 1.5% agarose gel which incorporated 0.007% w/v ethidium bromide and visualised by UV illumination. The CYP1B1 PCR product was sequenced, after purification, by the direct dideoxy sequencing technique with a T7 sequencing kit (Pharmacia, Milton Keynes, UK) used according to the manufacturer's protocol. To further investigate the relative amount of CYP1B1 mRNA in normal and tumour tissues, semi-quantitative RT-PCR of normal and tumour kidney samples was performed using serial dilution of cDNA (24). β -actin mRNA was used as an internal control (29).--